Abstract

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A (1→3,1→4)-β-glucan-specific monoclonal antibody and its use in the quantitation and immunocytochemical location of (1→3,1→4)-β-glucans

Peter J. Meikle[1], Nicholas J. Hoogenraad[2], Ingrid Bonig[3], Adrienne E. Clarke[3] and Bruce A. Stone[2,]*

Summary

Monoclonal antibodies were raised against a (1→3,1→4)-β-glucan-bovine serum albumin (BSA) conjugate. One antibody (BG1) selected for further characterization, was specific for (1→3,1→4)-β-glucan, displaying no binding activity against a (1→3)-β-glucan-BSA conjugate and minimal binding against a cellopentaose-BSA conjugate. A range of oligosaccharides was prepared by enzymatic digestion of (1→3,1→4)-β-glucan, purified by size exclusion chromatography and characterized by ^{1}H-NMR and anion exchange chromatography.

These (1→3,1→4)-β-oligoglucosides, together with (1→3)-β- and (1→4)-β-oligoglucosides were used to characterize the binding site of the monoclonal antibody (BG1) by competitive inhibition. The monoclonal antibody showed maximal binding to a heptasaccharide with the structure Glc(1→3) Glc(1→4) Glc(1→4) Glc(1→3) Glc(1→4) Glc(1→4) Glc and was determined to have an affinity constant of 3.8×10^4 M^{-1} for this oligoglucoside.

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PDF (3989KB)

Products

PRODUCTS FOR BOTANICAL MICROSCOPISTS AND POLYSACCHARIDE ENZYMOLOGISTS

Histochemical Reagents:

Aniline Blue Fluorochrome for callose
An analytical probe for (1-3)-beta-D-glucans (callose). Highly specific for (1-3)-beta-glucans [Evans *et al.* (1984) *Carbohydrate Polymers* **4:** 215-230; Stone *et al.* (1984) *Protoplasma* **122:** 191-195]. May be used for the quantitative determination of callose [Kauss (1989) *Plant Physiol.* **81:** 171-176] and in flourescence assays for (1-3)-beta-glucan synthase products.

Yariv Reagents for arabinogalactan-proteins
Specific probes for the detection of AGPs in tissue sections [Anderson *et al.* (1977) *Aust J. Plant Physiol.* **4:** 143-158]. May be used to detect and quantify AGPs in tissue extracts [Van Holst & Clarke (1985) Anal Biochem. 148: 446-450] and to detect AGPs in crossed-electrophoretic separations [Van Holst & Clarke (1986) *Plant Physiol.* **80:** 786-798].

Gum Arabic an arabinogalactan-protein
Gum Arabic AGP is used as a reference standard in many methods involving detection, quantification and analytical analyses of AGPs from plant tissues.

Immunohistochemical Reagents:

Monoclonal Antibodies (Murine)

to (1-3)-beta-D-glucan
No cross-reactivity with (1-4)-beta-D-glucans or (1-3;1-4)-beta-D-glucans [Meikle *et al.* (1991) *Planta* **188:** 1-8].

to (1-3;1-4)-beta-D-glucan
No cross-reactivity with (1-3)-beta-D-glucans [Meikle *et al.* (1994) *The Plant Journal* **5:** 1-9].

to (1-4)-beta-D-mannan and galacto-(1-4)-beta-D-mannan
[Pettolini *et al.* (2001) *Planta.* In press].

Each of these antibodies can be used with second stage, gold- or fluorochrome-labelled rabbit, anti-mouse antibody for immunohistochemical studies.

Enzymes:

(1-3;1-4)-beta-D-Glucan Hydrolase from *Bacillus subtilis* (EC 3.2.1.73).
Specifically hydrolyses beta-D-glucans containing both (1-3)- and (1-4)-beta-D-glucosidic linkages in linear sequences. Does not hydrolyse (1-4)-beta-D-glucans or (1-3)-beta-D-glucans [Anderson & Stone (1975) *FEBS Letters* **52:** 202-207].

Substrates:

Pachyman [(1-3)-beta-D-Glucan] ex *Poria cocus*
Useful as a positive control in fluoroescence microscopy studies on callose using the aniline blue fluorochrome or (1-3)-beta-D-glucan specific monoclonal antibody.

Carboxymethyl-pachyman [(1-3)-beta-D-Glucan]
(D.S. 0.31)
This water-soluble O-carboxymethyl ether of pachyman is a useful substrate for assay of (1-3)-beta-D-glucan *endo*-hydrolases either reductometrically or viscometrically.

(1-3;1-4)-beta-Glucan from Barley
A water-soluble polysaccharide from walls of barley endosperm cells. Useful as a substrate for (1-3;1-4)-beta-D-glucan hydrolase and as a control in immunohistochemical studies with the (1-3;1-4)-beta-D-glucan monoclonal antibody [Meikle *et al* (1994) *The Plant Journal* **5**: 1-9].

Arabino-(1-4)-beta-D-Xylan from wheat flour
A water-soluble polysaccharide from walls of wheat endosperm cells. Useful as a substrate for (1-4)-beta-D-xylan hydrolase.

Reference Text:

Chemistry and Biology of (1-3)-beta-glucans

By Professor Bruce A Stone and Professor Adrienne E Clarke

Glucans, with the (1-3)-beta-glucosidic linkage as a major feature, are present in most higher plants and many lower plants and microorganisms. They may occur as major structural or storage components or be formed at very specific sites in response to particular developmental events or stimuli. In many cases their functional role is a mystery, in others it is well established. Their distribution and physiological involvement indicates that they are important to fields such as agriculture and biotechnology, and may also have impact in medicine, throught their role in immunology and cancer therapy.

Contents:

The book is an invaluable reference handbook for not only graduate students and teachers in universities but also scientists in industrial, agricultural and medical research and develpment laboratories.

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Article

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Cell wall (1 → 3)- and (1 → 3, 1 → 4)-β-glucans during early grain development in rice (Oryza sativa L.)

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Abstract:

Abstract. Immunogold labeling was used to study the distribution of (1vMv3)-g-glucans and (1vMv3, 1vMv4)-g-glucans in the rice grain during cellularization of the endosperm. At approximately 3-5 d after pollination the syncytial endosperm is converted into a cellular tissue by three developmentally distinct types of wall. The initial free-growing anticlinal walls, which compartmentalize the syncytium into open-ended alveoli, are formed in the absence of mitosis and phragmoplasts. This stage is followed by unidirectional (centripetal) growth of the anticlinal walls mediated by adventitious phragmoplasts that form between adjacent interphase nuclei. Finally, the periclinal walls that divide the alveoli are formed in association with centripetally expanding interzonal phragmoplasts following karyokinesis. The second and third types of wall are formed alternately until the endosperm is cellular throughout. All three types of wall that cellularize the endosperm contain (1vMv3)-g-glucans but not (1vMv3, 1vMv4)-g-glucans, whereas cell walls in the surrounding maternal tissues contain considerable amounts of (1vMv3, 1vMv4)-g-glucans with (1vMv3)-g-glucans present only around plasmodesmata. The callosic endosperm walls remain thin and cell plate-like throughout the cellularization process, appearing to exhibit a prolonged juvenile state.

Keywords:

Key words: Cell wall (endosperm) · Development (cereal grain) · Endosperm (development · structure) · Glucan (immunolocation) · Grain development · Oryza (cell wall)

Problems caused by barley beta-glucans in the brewing industry.

McCleary, B.V. (1986). **Chemistry in Australia, 53**, 306-308.

Brewing, the oldest application of biotehcnology, is now a mix of trade art and modern science. This article describes new applications of enzyme chemistry to trouble-shooting in beer production.

What are Barley β-glucans?

Barley β-glucans are unbranched polymers of β-linked D-glucosyl residues. They are also referred to as mixed-linkage β-glucans, (1-3)(1-4)-β-D-glucans or barley gums. These β-glucans form a major part of the cell-walls in barley endosperm tissue, representing about 75% of total cell-wall carbohydrate.

Barley β-glucan extracted by water at 40°C is generally considered to be composed predominantly of cellotriosyl and cellotetraosyl residues separated by single (1-3)-β-linkages (refer to Scheme 1).

Small, but significant, proportions of longer blocks of up to 10 contiguous (1-4)-β-linkages are also present. Contiguous (1-3)-β-linkages would appear to be absent in the water-soluble glucan extracted from barley but have been reported to occur in β-glucan extracted from malt.

Problems caused by barley β-glucans

During the malting of barley grain, β-glucan and other major storage components are partially degraded by enzymes synthesised *de novo*. The major enzymes responsible for depolymerisation of the barley β–glucan, namely malt β-glucanases, rapidly increase in amount during the malting process and generally give effective removal of polymeric barley β-glucan.

If β-glucan is fully degraded to glucose during malting and mashing it contributes to fermentable sugars ("extract"). However, if this glucan is not fully degraded there is a loss of extract and high-molecular-weight, viscous material can be released into solution.

Loss of extract may be attributed both to incomplete degradation of β-glucan and also to the fact that sufficient cell-wall material may remain to provide a physical barrier to digestion of cell contents, particularly starch.

This problem is accentuated with modern, rapid, malting processes where the grain can be very unevenly modified by endogenous enzymes. Also, where unmodified barley adjunct is used as a less expensive source of carbohydrate it is essential to supply sufficient and suitable enzymes, from malt or otherwise, to ensure β-glucan breakdown.

Barley β-glucan is composed of both "soluble" and "insoluble" components and it is considered that it is the insoluble component which causes most problems in the brewing industry. The reasons for solubility or insolubility have not been clearly defined.

Some researcher consider that insolubility may be due to binding of the β-glucan through peptide linkages to protein, whereas others consider it is due to structural variation in the glucan itself, ie barley β-glucan subfractions with a high proportion of regions of sequential (1-4)-β-linked D-glucosyl residues could be expected to have a more cellulose-like nature and thus be less soluble.

In contrast, our results indicate that there is no major difference between the soluble and insoluble fractions, although, with some barley flours we have found that the more readily extractible β-glucan has a lower molecular size.

1: Plant J. 1994 Jan;5(1):1-9. Related Articles, Links

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